OMEGA bio-tek

Innovations in nucleic acid isolation

Product Manual

E.Z.N.A.® BAC/PAC DNA Kit

D2156-00	5 preps
D2156-01	50 preps

May 2013

For research use only.Not intended for diagnostic testing.

E.Z.N.A.® BAC/PAC DNA Kit

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Introduction and Overview

The E.Z.N.A.® BAC/PAC DNA Kit is designed for rapid high-throughput purification of BACs, PACs, and P1s from small volume bacterial cultures. This kit is based on a modified alkaline lysis procedure that has been adapted for use with spin columns and high-throughput procedures. The protocol has been tested using a variety of low copy cosmids, BACs, PACs, P1s, and *E. coli* strains. In addition this kit can also be used for high copy plasmid isolation. Two protocols are provided in this handbook for your convenience. The new standard protocol provides a fast and reliable method for purification of BAC, PAC, P1, and plasmid DNA using a HiBind® DNA Mini Column. The second protocol is for the isolation of low copy number cosmids, BACs, PACs, and P1s.

New in this Edition:

- This manual has been edited for content and redesigned to enhance user readability.
- Equilibration Buffer is no longer included with this kit. An optional Column Equilibration Protocol has been added to the protocol for your convenience.
- Equilibration Buffer is replaced with 3M NaOH provided by the user.

Kit Contents

Product	D2156-00	D2156-01
Purifications	5	50
HiBind® DNA Mini Columns	5	50
2 mL Collection Tubes	5	50
T1 Buffer	5 mL	20 mL
T2 Buffer	5 mL	20 mL
T3 Buffer	5 mL	20 mL
BAC Binding Buffer	1.5 mL	5 mL
Elution Buffer	1.0 mL	10 mL
SPM Wash Buffer	3 mL	15 mL
Linear Polyacrylamide	15 µL	120 µL
RNase A	50 µL	100 µL
User Manual	✓	✓

* 10 mM Tris-HCl, pH 8.5

Storage and Stability

All of the E.Z.N.A.® BAC/PAC DNA Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. RNase A should be stored at 2-8˚C. T1 Buffer (once RNase A is added) should be stored at 2-8˚C. All remaining components should be stored at room temperature.

1. Add the vial of RNase A to bottle of T1 Buffer. Store at 4°C.

2. Dilute BAC Binding Buffer with isopropanol as follows and store at room temperature.

Kit	Isopropanol to be Added
D2156-00	4.5 mL
D2156-01	15 mL

3. Dilute SPM Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D2156-00	7 mL
D2156-01	35 mL

Yield and Quality of DNA

Determine the absorbance of an appropriate dilution (20- to 50-fold) of the sample at 260 nm and then at 280 nm. The DNA concentration is calculated as follows:

DNA concentration = Absorbance 260 × 50 × (Dilution Factor) µg/mL

A ratio greater than 1.8 indicates greater than 90% nucleic acid. Alternatively, quantity (as well as quality) sometimes can be determined best by agarose gel/ethidium bromide electrophoresis by comparison to DNA samples of known concentrations. Typically, the majority of the DNA eluted is in monomeric supercoil form, though concatemers may also be present.

E.Z.N.A.® BAC/PAC DNA Kit - Standard Protocol

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 18,000 x g
- Vortexer
- Ice bucket
- Nuclease-free 1.5-2 mL microcentrifuge tubes
- 10-15 mL culture tubes
- 70% Ethanol
- Isopropanol
- Sterile Deionized Water (or TE Buffer)

Before Starting:

- Prepare T1 Buffer, BAC Binding Buffer, and SPM Wash Buffer according to the Preparing Reagents section on Page 4
- Prepare an ice bucket
- Chill T3 Buffer on ice

1. Isolate a single colony from a freshly streaked selective plate and inoculate a culture of 2-5 mL LB or YT medium containing the appropriate antibiotic. Incubate for ~20-24 hours at 37°C with vigorous shaking (~300 rpm). Use a flask with a volume at least 4 times the culture volume.

2. Centrifuge 1.5-5 mL culture at 3,500-5,000 x *g* for 3 minutes at room temperature.

3. Decant or aspirate and discard the culture media.

   Note: To ensure that all traces of the medium are removed, use a clean paper towel to blot excess liquid from the wall of the vessel.

4. Add 200 µL T1 Buffer/RNase A. Vortex or pipet up and down to mix thoroughly. Complete resuspension of cell pellet is vital for obtaining good yields.

   Note: RNase A must be added to T1 Buffer before use. Please see the instructions in the Preparing Reagents section on Page 4.

5. Add 200 µL T2 Buffer. Invert and gently rotate the tube 10-20 times to obtain a clear lysate.

   Note: Avoid vigorous mixing as this will shear chromosomal DNA and lower BAC purity. Do not allow the lysis reaction to proceed more than 5 minutes. Store T2 Buffer tightly capped when not in use to avoid acidification from CO_2 in the air.

6. Let sit for 5 minutes at room temperature.

7. Add 200 µL cold T3 Buffer. Immediately invert several times until a flocculent white precipitate forms.

   Note: It is vital that the solution is mixed thoroughly and immediately after the addition of T3 Buffer to avoid localized precipitation. If the mixture still appears viscous, brownish, or conglobated, more mixing is required to completely neutralize the solution.

8. Incubate on ice for 5 minutes.

9. Centrifuge at maximum speed for 10 minutes at 4°C .

10. Transfer the cleared supernatant to a new 1.5 mL microcentrifuge tube (not provided).

11. Add 200 µL BAC Binding Buffer. Invert the tube 3-5 times to mix thoroughly.

    Note: BAC Binding Buffer must be diluted with isopropanol prior to use. Please see Page 4 for instructions.

12. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube.

Optional Protocol for Column Equilibration:

1. Add 100 µL 3M NaOH to the HiBind® DNA Mini Column.
2. Centrifuge at maximum speed for 30-60 seconds.
3. Discard the filtrate and reuse the collection tube.

13. Transfer the cleared supernatant from Step 10 to the HiBind® DNA Mini Column. Be careful not to disturb the pellet and that no cellular debris is transferred to the HiBind® DNA Mini Column.

14. Centrifuge at maximum speed for 30 seconds.

15. Discard the filtrate and reuse the collection tube.

16. Add 750 µL SPM Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

17. Centrifuge at maximum speed for 1 minute.

18. Discard the filtrate and reuse the collection tube.

19. Centrifuge the empty HiBind® DNA Mini Column for 2 minutes at maximum speed to dry the column matrix.

    Note: It is important to dry the HiBind® DNA Mini Column matrix before elution. Residual ethanol may interfere with downstream applications.

20. Transfer the HiBind® DNA Mini Column to a clean 1.5 mL microcentrifuge tube.

21. Add 30-50 µL Elution Buffer or sterile deionized water directly to the center of the column membrane.

    Note: The efficiency of eluting DNA from the HiBind® DNA Mini Column is dependent on pH. If using sterile deionized water, make sure that the pH is around 8.5.

22. Let sit at room temperature for 5 minutes.

23. Centrifuge at maximum speed for 2 minutes.

24. Store DNA at -20°C.

E.Z.N.A.® BAC/PAC DNA Kit - Low Copy Number Protocol

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 18,000 x g
- Vortexer
- Ice bucket
- Nuclease-free 1.5-2 mL microcentrifuge tubes
- 10-15 mL culture tubes
- 70% Ethanol
- Isopropanol
- Sterile Deionized Water (or TE Buffer)

Before Starting:

- Prepare T1 Buffer, BAC Binding Buffer, and SPM Wash Buffer according to the Preparing Reagents section on Page 4
- Prepare an ice bucket
- Chill T3 Buffer on ice

1. Isolate a single colony from a freshly streaked selective plate and inoculate a culture of 2-5 mL LB or YT medium containing the appropriate antibiotic. Incubate for ~20-24 hours at 37°C with vigorous shaking (~300 rpm). Use a flask with a volume at least 4 times the culture volume.

2. Centrifuge 1.5-5 mL culture at 3,500-5,000 x *g* for 3 minutes at room temperature.

3. Decant or aspirate and discard the culture media.

   Note: To ensure that all traces of the medium are removed, use a clean paper towel to blot excess liquid from the wall of the vessel.

4. Add 260 µL T1 Buffer/RNase A. Vortex or pipet up and down to mix thoroughly. Complete resuspension of cell pellet is vital for obtaining good yields.

   Note: RNase A must be added to T1 Buffer before use. Please see the instructions in the Preparing Reagents section on Page 4.

5. Add 260 µL T2 Buffer. Invert and gently rotate the tube 10-20 times to obtain a clear lysate.

   Note: Avoid vigorous mixing as this will shear chromosomal DNA and lower BAC purity. Do not allow the lysis reaction to proceed more than 5 minutes. Store T2 Buffer tightly capped when not in use to avoid acidification from CO_2 in the air.

6. Let sit for 5 minutes at room temperature.

7. Add 260 µL cold T3 Buffer. Immediately invert several times until a flocculent white precipitate forms.

   Note: It is vital that the solution is mixed thoroughly and immediately after the addition of T3 Buffer to avoid localized precipitation. If the mixture still appears viscous, brownish, or conglobated, more mixing is required to completely neutralize the solution.

8. Incubate on ice for 5 minutes.

9. Centrifuge at maximum speed for 10 minutes at 4°C .

10. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube.

11. Transfer the cleared supernatant from Step 10 to the HiBind® DNA Mini Column. Be careful not to disturb the pellet and that no cellular debris is transferred to the HiBind® DNA Mini Column.

12. Centrifuge at maximum speed for 1 minute.

13. Discard the HiBind® DNA Mini Column. **Do not discard the filtrate!**

14. Add 2 µL linear polyacrylamides to the 2 mL Collection Tube containing the cleared cell lysate.

15. Add 0.7 volumes isopropanol to the samples. Vortex at maximum speed for 15 seconds.

    Note: For example, add 546 µL isopropanol to 780 µL cell lysate.

16. Centrifuge at maximum speed for 10 minutes at room temperature.

17. Carefully aspirate and discard the supernatant, making sure not to dislodge the DNA pellet.

18. Add 500 µL 70% ethanol.

19. Centrifuge the 2 mL collection tube containing pellet (in the same orientation as before) for 10 minutes.

20. Carefully aspirate and discard the supernatant, making sure not to dislodge the DNA pellet. Invert the tube containing the DNA pellet on a paper towel for 10-15 minutes to air dry the DNA pellet.

    Note: Ensure that no alcohol droplets are visible after air drying. Do not over dry the DNA pellet. Over drying the DNA pellet will make redissolving the pellet difficult.

21. Add 30-50 µL Elution Buffer.

22. Let sit overnight at room temperature.

23. Store DNA at -20°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **1-800-832-8896**.

Problem	Cause	Solution
Low DNA yields	Poor cell lysis	Only use LB or YT medium containing ampicillin. Do not use more than 5 mL culture with the standard protocol.
		Cells may not have been dispersed adequately prior to the addition of T2 Buffer. Make sure to vortex cell suspension to completely disperse.
		Increase incubation time with T2 Buffer to obtain a clear lysate.
	Bacterial colony is not fresh	T2 Buffer if not tightly closed, may need to be replaced.
		Use fresh glycerol cultures and avoid repeated freeze/thaw cycles. Always make enough replica plates and use precultures for inoculation. Any remaining precultures can be used to set up fresh glycerol stocks.
Problem	**Cause**	**Solution**
No DNA Eluted	Lysate prepared incorrectly	Check the stock of buffers and age of the buffers. Make sure that the correct volume of buffer has been added to the sample.
	T2 Buffer precipitated	Warm up the T2 Buffer to dissolve the precipitate.
	Cells are not completely resuspended	Pelleted cells should be completely resuspended with T1 Buffer. Do not add T2 Buffer until an even cell suspension is obtained.

Troubleshooting Guide

Problem	Cause	Solution
High molecular weight DNA contamination of product	Over mixing of cell lysate upon addition of T2 Buffer	Do not vortex or mix aggressively after adding T2 Buffer.
	Culture overgrown	Overgrown culture contains lysed cells and degraded DNA. Do not grow cell for longer than 16 hours.
Problem	**Cause**	**Solution**
DNA degraded after storage	High levels of endonuclease activity	Perform the heat inactivation step.
Problem	**Cause**	**Solution**
RNA visible on agarose gel	RNase A not added to T2 Buffer	Add 1 vial of RNase to each bottle of T2 Buffer.
	DNA floats out of well while loading agarose gel	Air dry the DNA pellet before redissolving the DNA.

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
DNase/RNase-free microcentrifuge tubes, 1.5 mL, 500/pk, 10 pk/cs	SSI-1210-00
DNase/RNase-free microcentrifuge tubes, 2.0 mL, 500/pk, 10 pk/cs	SSI-1310-00
HiBind® DNA Mini Columns (200)	DNACOL-02
T2 Buffer (60 mL)	PS007
Elution Buffer (100 mL)	PDR048
SPM Wash Buffer (40 mL)	PS014

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Notes: